AN ACCELERATED, ACTIVELY MIXED, REUSABLE DYNAMIC ARRAY™ FOR FLUIDIGM BIOMARK™ AND EP1™ SYSTEMS

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ABSTRACT

We present here a reusable microfluidic device for high throughput polymerase chain reaction (PCR) with an accelerated workflow.

By dilation pumping, the array can be cleaned with washing buffer after each use. The washing buffer is replaced with DNA free water by the same means before an overnight bake-dry step. Arrays have been used up to 15 times.

To accelerate the mixing speed, the chips use active mixings. After sample and assay were loaded, the active valve was pushed and released alternatively with a frequency of 0.2Hz. The mixing speed, therefore, was dramatically accelerated from 70min to 12 min.

KEYWORDS

Accelerated, active mixing, reuse, High throughput, dynamic array, real-time PCR, Genotyping.

INTRODUCTION

Fluidigm has previously demonstrated integrated fluidic circuits (IFCs) arrays with thousands of reaction chambers, which can be loaded within an IFC controller in about two hours. The arrays are then transferred to Fluidigm BioMark™ or EP1™ system for thermal cycling for 1.5 hours. The real-time or end-point fluorescence readings are then recorded for data analysis.

The IFCs described here greatly shorten the loading and PCR cycling time, and cut down the operation cost by allowing multiple uses of consumables. These combined characteristics dramatically reduce the cost per data point for researchers pursuing higher sample throughput.

EXPERIMENT

The microfluidic chip was built by a typical Multilayer Soft Lithography (MSL) processing. Two negative structured molds were first constructed with positive and negative photo resists on Silicon wafer substrates. Then the structures were transferred to a spin coated PDMS control layer and a cast PDMS reaction layer. The thicknesses of control layer and reaction layer were 20 μm and 4 mm, respectively. The two layers were then aligned and bonded together after oxygen plasma treatment. The channels at different layers were connected by laser punched vias. A detailed description of chip fabrication can be found in reference. [1]

The real-time PCR and genotyping assays contain both forward and reverse primers and FAM and VIC dye labeled cleavage probes (TaqMan probes, Applied Biosystems).

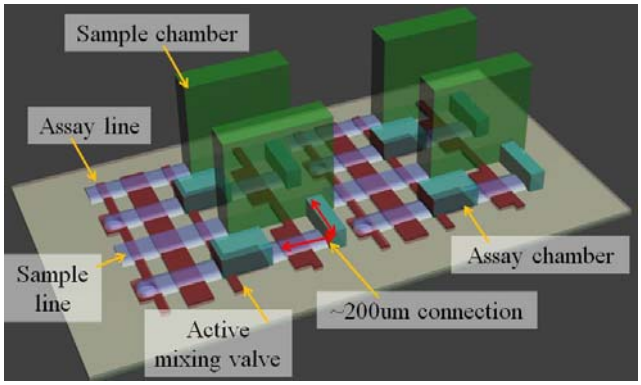

Figure 1. The 3D view of 4 unit cell structures

A 3D CAD drawing of unit cell is shown in Figure 1, where an active mixing valve was put under the assay chamber. In this 4,608 reactor design, the reaction and assay chambers are connected with a long (200um), narrow (80um) and shallow (8um) channel. After sample and assay were loaded, the active valve was pushed and released alternatively. The mixing efficiency, therefore, was dramatically accelerated due to Taylor dispersion. [2]

Figure 2 illustrates the mixing efficiency changes along the mixing time for active mixing and diffusion mixing with the same array design. The mixing time at 90% level was observed to be 12 minutes for active mixing strategy; the mixing time for diffusion mixing alone was 70 minutes, which is consistent to the estimations. [3]

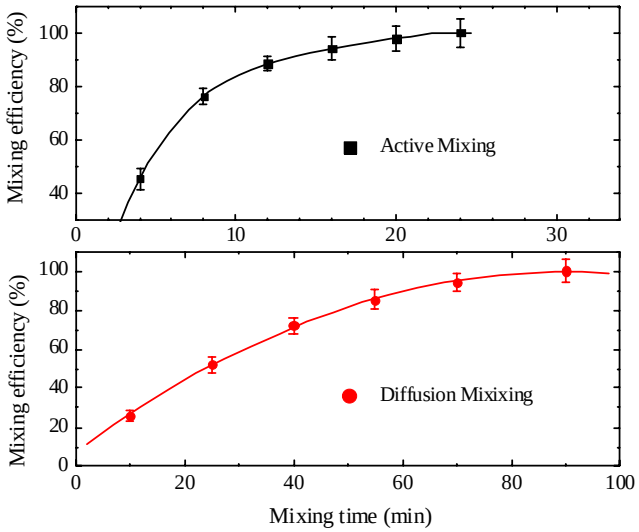

Figure 2. Mixing efficiency changes versus mixing time

The IFC arrays were designed to be washable. As shown in Figure 3(a), there are two dilation pump valves guarding upstream and downstream of sample lines. During a charging step, the upstream valve was open while downstream valve was closed. The washing buffer was loaded into elastomer array with expanded volume. Then both valves were closed to assure an even pressure distribution, as shown in Figure 3(b). Next, as in Figure 3(c), the upstream valve kept closed and the downstream valve was opened so the washing buffer could be released from the chip. Thereafter, both valves were closed, as shown in Figure 3(d), ready for a new cycle. By dilation pumping, the used array can be cleaned with washing buffer. The washing buffer can be washed away before an overnight bake-dry step.

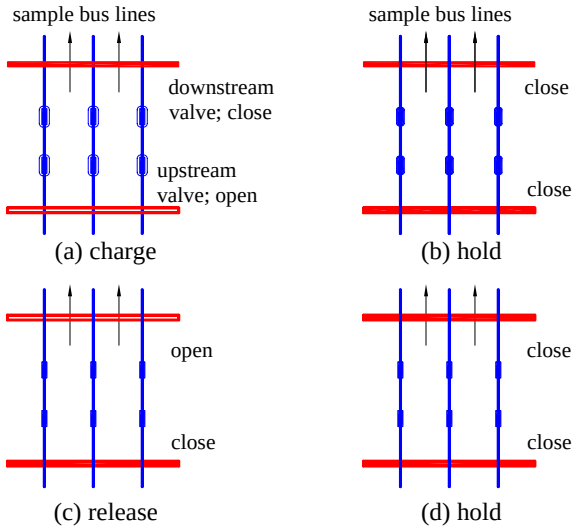

Figure 3. Illustration of washing IFC

To verify the chip performances after recovery, real-time PCR was used to check the possible presence of amplicon from the previous reaction. The PCR Ct heat maps in two sequential chip runs are shown in Figure 4. The positive and negative reaction cocktails were alternatively loaded to detect any copies as target left behind. Although PCR in nano volumes reliably amplifies even a single copy of template, our observed rate of false positives was less than 0.025%, demonstrating complete removal of amplicon from the previous reactions. These arrays, hosting 48 samples by 48 assays, have been used up to 15 times.

Figure 5 shows the SNP Genotyping clusters from an accelerated, reusable array. This prototype array runs 192 samples and 24 assays. Observed call rates are >99% and accuracies are >99.75%. The accelerated workflow delivers 4,608 SNP data from sample to answer in one hour, which includes half hour sample loading and half hour thermal cycling.

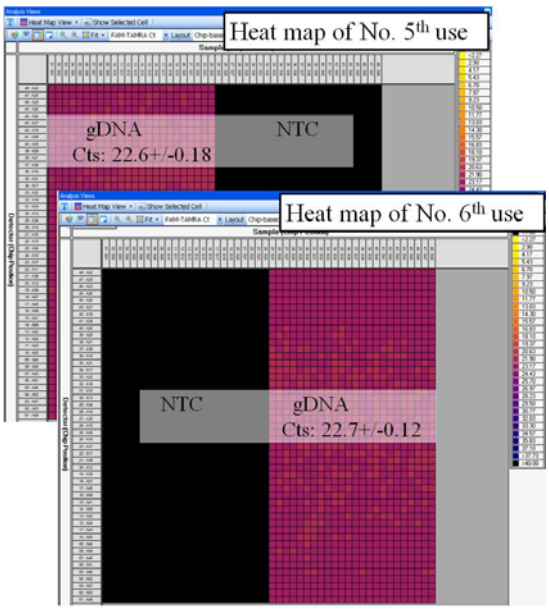

Figure 4. Two real-time PCR Ct heat maps in continuous use of same reusable array. In the following cycling, the right half of chip, which was loaded with positive reactions, has shown successfully recovered from previous use. And the left half of chip, which was loaded negative reactions, has demonstrated no amplicon carryover from previous chip run.

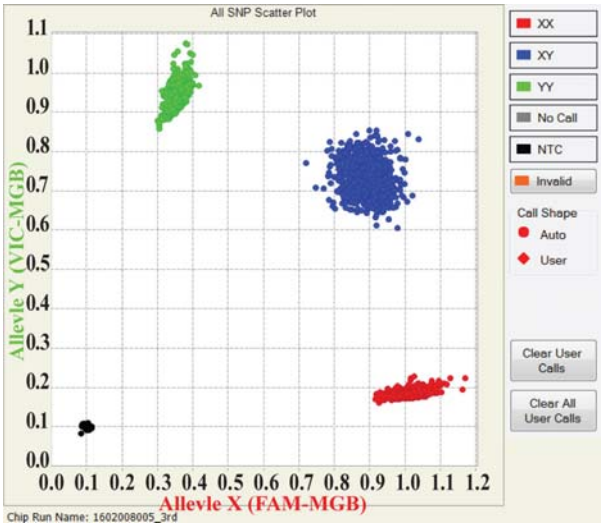

Figure 5. SNP Genotyping allele clusters from an accelerated, reusable IFC

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